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(54) Title: METHOD FOR TREATING A MAMMAL BY ADMINISTRATION OF A COMPOUND HAVING THE ABILITY TO RELEASE CO

(57) Abstract: The present invention relates to molybdenum carbonyl complexes useful for inhibiting tumor necrosis factor (TNF) production and for treating inflammatory diseases.

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# METHOD FOR TREATING A MAMMAL BY ADMINISTRATION OF A COMPOUND HAVING THE ABILITY TO RELEASE CO

[0001] This application is a continuation-in-part of U.S. Patent Application No. 11/453,319, filed June 14, 2006, which is a divisional application of U.S. Application No. 11/288,670, filed November 29, 2005, which is a divisional application of U.S. Application No. 10/356,738 (now U.S. Patent No. 7,011,854), filed February 3, 2003, which is based on and claims the benefit of U.S. Provisional Application No. 60/353,233, filed February 4, 2002. This application also claims the benefit of U.S. Provisional Application No. 60/752,571, filed December 20, 2005. The entire disclosures of these applications are relied upon and incorporated herein by reference. U.S. Patent No. 7,011,854 is relied upon and incorporated herein by reference.

[0002] Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

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## Field of the Invention

[0004] The molybdenum carbonyl complexes described herein are useful for inhibiting tumor necrosis factor (TNF) production and for treating inflammatory diseases.

## Background of the Invention

[0005] The treatment of acute and chronic inflammatory diseases remains a major challenge. Rheumatoid arthritis is an example of a chronic inflammatory disease for which current treatment is inadequate. The traditional drugs in current use are nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and various disease-modifying antirheumatic drugs (DMARDs). These drugs are effective only in a subset of patients and their long term use is limited by side effects, some of which are severe.

[0006] A major advance in the treatment of rheumatoid arthritis came with the introduction of tumor necrosis factor antagonists. These drugs, either antibodies or engineered soluble receptors that bind TNF, have improved the treatment of rheumatoid arthritis (1, numbers in parenthesis refer to numbered references at the end of this patent

application) and are also useful in a variety of other inflammatory conditions (2-6). A drawback of these DMARDs is that their production is very expensive. Moreover, their long term use is also associated with side effects, some of which are severe (7). However, TNF antagonism is a validated strategy for treating rheumatoid arthritis and other inflammatory conditions (8).

**[0007]** Efforts are currently under way to develop small molecular weight TNF inhibitors that can be produced at low cost and that may have fewer side effects by acting locally in inflamed tissues. One strategy to achieve this goal is through the use of endogenously produced, small molecular weight substances that are known to inhibit TNF production. One such molecule is carbon monoxide (CO). CO inhibits TNF production *in vitro* and *in vivo* and has shown impressive anti-inflammatory effects in animal models (9, 10). In addition to inhibiting TNF production, CO has additional anti-inflammatory effects. It inhibits the production of other proinflammatory cytokines such as IL-1, IL-6 and MIP-1 (11, 12), enhances IL-10 production (11), inhibits excessive NO production by inducible nitric oxide synthase (13), inhibits mast cell activation (14), and modulates immune responses (15). Exogenous CO may also induce the expression of hemoxygenase-1 (HO-1) either by the transient generation of reactive oxygen species (16) or via the enhancement of IL-10 production (17). HO-1 is known to have a wide variety of protective functions (18), most of which are mediated by its products CO and biliverdin/bilirubin. Thus, the beneficial effects of exogenous CO may be further augmented by the induction of endogenous CO and biliverdin/bilirubin production.

**[0008]** CO inhalation has been a very useful experimental procedure to reveal the beneficial effects of CO in animal disease models. Several patent applications disclose the use of CO as a gas for a wide variety of indications associated with inflammatory reactions (US 2002155166, US 2003039638, US 2003219496, US 2003219497, US 2004052866, WO 03/103585, WO 04/043341). However, CO administration by inhalation is not practical for clinical applications, as it requires special delivery devices such as ventilators, face masks, tents, or portable inhalers. Moreover, CO delivery to therapeutic targets by inhalation is inefficient, because it involves transport of CO by hemoglobin. Hemoglobin binds CO reversibly, but with very high affinity. Therefore, the doses required to deliver CO to therapeutic targets in diseased tissues are likely to be associated with adverse effects.

**[0009]** CO releasing molecules (CORMs) that can deliver CO directly to therapeutic targets without the formation of intermediate CO-hemoglobin complexes have also been developed (19, 20). Impressive, therapeutic effects have been achieved with ruthenium-

based CORMs in tissue culture (16), a perfused heart model (20) and *in vivo* in myocardial infarction models (21). Ruthenium-based CORMs have also been shown to inhibit TNF and excessive NO production in tissue culture (16). A wide variety of CORMs have been disclosed for their use in the treatment of inflammatory diseases and diseases associated with acute or chronic inflammatory reactions (WO 02/092075, WO 04/045598, WO 04/045599, WO 02/078684, US 2004/067261). The potential advantage of CO delivery by CORMs over CO delivery by inhalation is generally recognized. However, CORMs should be able to deliver CO selectively to diseased tissues. The identification of CORMs that are best suited for the treatment of a particular disease remains a major challenge of CORM development. Very little is presently known about chemical reactions of organometallic carbonyl complexes in aqueous solutions.

**[0010]** The present invention is directed to these and other important ends.

## Summary of the Invention

**[0011]** In one embodiment, methods for inhibiting tumor necrosis factor production in an animal in need thereof are described herein. The methods include administering to the animal an effective amount of a compound of the Formula I:

$$[Mo(CO)_5Y]Q$$

**I**

wherein Y is bromide, chloride or iodide; and

Q is $[NR^{1-4}]^+$

where $R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

**[0012]** In one embodiment, methods for inhibiting tumor necrosis factor production in a cell are described herein. The methods include contacting the cell with a compound of Formula I.

**[0013]** In one embodiment, methods for treating or preventing a disease in an animal in need thereof are described herein. The methods include administering to the animal an effective amount of a compound of Formula I.

**[0014]** In one embodiment, CO releasing molecules that are useful for the treatment of inflammatory diseases, including without limitation rheumatoid arthritis are described herein.

## Brief Description of the Figures

**[0015]** **Figure 1** depicts the apparatus used to detect spontaneous CO release from Compound I.1.

**[0016]** **Figure 2** demonstrates the toxicity of Compound I.1 in RAW264.7 cells at 2 hours, 4 hours, and 24 hours using the MTT assay.

**[0017]** **Figure 3** demonstrates CO release *in vivo* of Compound I.1. Three doses were used and the CO-hemoglobin levels were measured at 0, 30, 120 and, in one case, 330 minutes.

**[0018]** **Figure 4** demonstrates the inhibition of lipopolysaccharide (LPS)-induced TNF production by intraperitoneal application of various doses of Compound I.1.

**[0019]** **Figure 5** demonstrates the inhibition of LPS-induced lethal effects of lipopolysaccharide.

**[0020]** **Figures 6A-6B** demonstrate the average left (**Figure 6A**) or right (**Figure 6B**) paw volume in an adjuvant arthritis model in rats of the control, positive control (methylene chloride)-treated and Compound I.1-treated groups.

**[0021]** **Figures 7A-7B** demonstrate the average left (**Figure 7A**) or right (**Figure 7B**) paw circumference in an adjuvant arthritis model in rats of the control, positive control (methylene chloride)-treated and Compound I.1-treated groups.

**[0022]** **Figure 8** demonstrates the arthritis index in an adjuvant arthritis model in rats of the control, positive control (methylene chloride)-treated and Compound I.1-treated groups.

**[0023]** **Figure 9** demonstrates CO release *in vivo* of Compound I.1 at a concentration of 100 mg/kg. The CO-hemoglobin levels were measured at time intervals.

**[0024]** **Figure 10** demonstrates the *in vivo* release of CO from Compound I.1 encapsulated in TRIMEB.

## Detailed Description of the Invention

**[0025]** In one embodiment, methods for inhibiting tumor necrosis factor production in an animal in need thereof are described herein. The methods include administering to the animal an effective amount of a compound of the Formula I:

$$[Mo(CO)_5Y]Q$$

**I**

wherein Y is bromide, chloride or iodide; and

Q is $[NR^{1\text{-}4}]^+$

where $R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

**Definitions**

**[0026]** As used herein, the term "alkyl" means a $C_1$-$C_{12}$ saturated hydrocarbon chain, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, or n-dodecyl. In one embodiment, alkyl is a $C_1$-$C_6$ or a $C_1$-$C_4$ saturated hydrocarbon chain.

**[0027]** As used herein, the term "animal" includes, without limitation, a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, monkey, chimpanzee, baboon, or rhesus. In one embodiment, the animal is a mammal. In another embodiment, the animal is a human.

**[0028]** As used herein, the term "halide" means fluoride, chloride, bromide, or iodide.

**[0029]** As used herein, the term "spontaneous release" means release by a thermal, chemical, oxidative, or photodynamic process.

**[0030]** As used herein, the term "release by metabolic process" means release with the involvement of one or more enzymes, such as cytochrome P450 or glutathione S-transferase.

**[0031]** As used herein, the "CO" means carbon monoxide; "CORM" means carbon monoxide releasing molecule; "DMARDS" means disease-modifying antirheumatic drugs; "LPS" means lipopolysaccharide; "n-Bu" means n-butyl; "n-Pr" means n-propyl; "NSAID" means nonsteroidal anti-inflammatory drugs; and "TNF" means tumor necrosis factor.

**Compounds of Formula I**

**[0032]** In one embodiment, the present compounds of the Formula I are described herein:

$$[Mo(CO)_5Y]Q$$

I

wherein Y is bromide, chloride or iodide; and

Q is $[NR^{1\text{-}4}]^+$

where $R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

**[0033]** The compounds of Formula I provide convenient stability under air at room temperature to allow easy manipulation. Moreover, the compounds of Formula I provide the advantage of improved stability and solubility in water, including under the acidic pH range found, for example, in the gastric fluid. Without wishing to be bound by theory, applicants believe that this stability derives from the lower basicity of the halide anion.

**[0034]** The compounds of Formula I bearing a tetraalkylammonium cation also provide improved stability in water at physiologic pH relative to their analogues with alkaline cations, even when such an alkaline cation is stabilized by a cyclic or acyclic chelating polyether. Again without wishing to be bound by theory, applicants believe that this stability in water

derives at least in part from the favorable cation-anion interaction provided by a tetraalkylammonium cation.

**[0035]** In addition, the compounds of Formula I provide enhanced release of carbon monoxide, for example, in response to attack by radical oxygen species, relative to thermally induced carbon monoxide release (substitution) in the absence of such species. Since the onset of this release is very facile, the compounds of Formula I also provide efficient release of carbon monoxide at an inflammatory site in an animal where radical oxygen species can be generated or accumulated in biologically elevated concentrations.

**[0036]** In some embodiments, Y is bromide or chloride.

**[0037]** In other embodiments, in a compound of Formula I, Y is bromide.

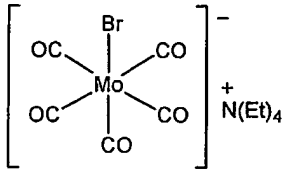
**[0038]** In still other embodiments, Y is iodide.

**[0039]** In further embodiments, Q is a tetraethylammonium cation, a tetra(n-butyl)ammonium cation, a tetra(n-propyl)ammonium cation, a tetra(i-propyl)ammonium cation or a tetramethylammonium cation.

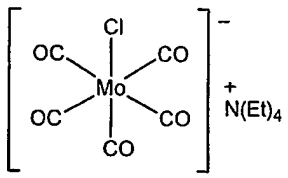
**[0040]** In other embodiments, Q is a tetraethylammonium cation.

**[0041]** In some embodiments, $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_{12})$-alkyl. In other embodiments, $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_8)$-alkyl. In further embodiments, $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_6)$-alkyl. In yet other embodiments, $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_4)$-alkyl.

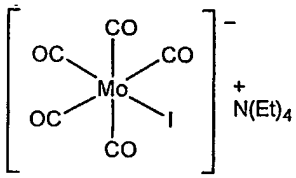
**[0042]** In one embodiment, the compound of Formula I is one of the following compounds:




I.1




I.2

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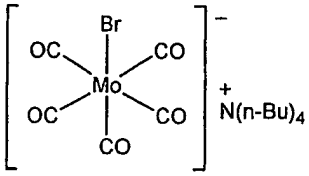


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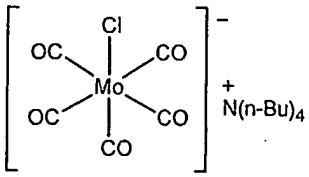
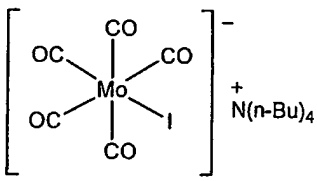
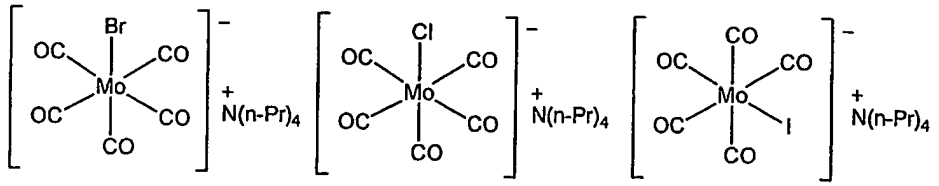


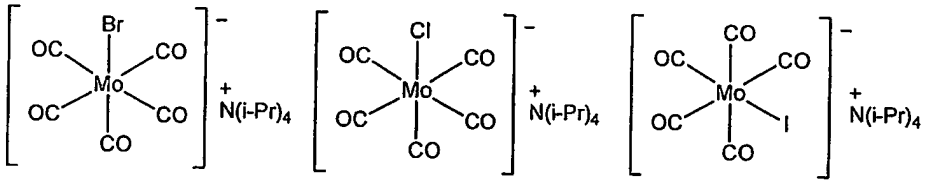

I.5



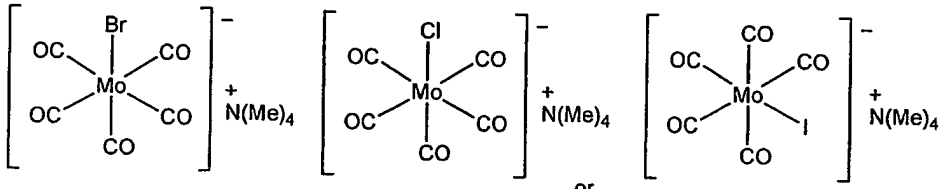

I.6




I.7 I.8 I.9

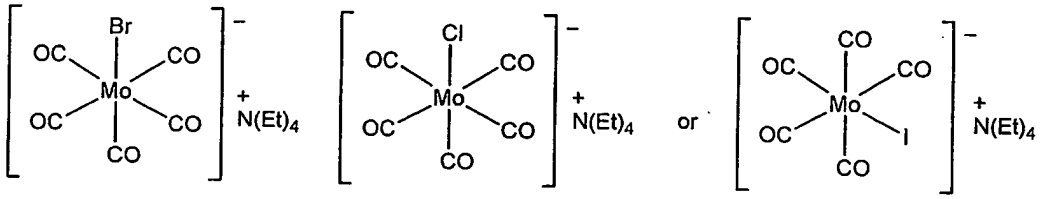



I.10 I.11 I.12



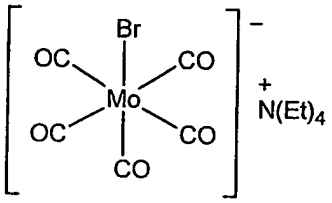

I.13 I.14 or I.15

**[0043]** In another embodiment, the compound of Formula I is one of the following compounds:




I.1 I.2 I.3

**[0044]** In another embodiment, the compound of Formula I is




I.1

## Methods of Making Compounds of Formula I

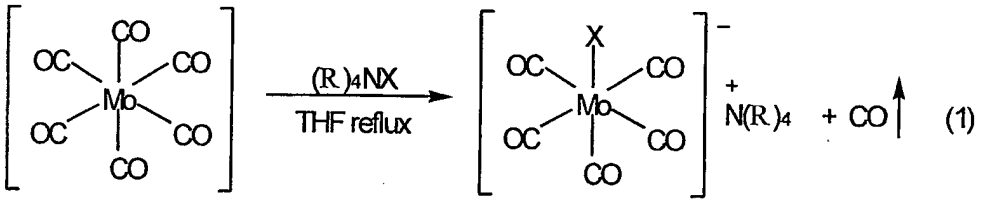
**[0045]** The compounds described herein can be prepared using a variety of methods well known in the art of molybdenum organometallic chemistry. The common starting material is $Mo(CO)_6$ that is commercially available or accessible from other Mo salts through known

procedures. Tetralkylammonium halides are usually commercially available or can be prepared by alkylation of the corresponding amines, which are also commercially available. General synthetic routes to many of the compounds described herein are known in the art of molybdenum organometallic chemistry as follows.

**[0046]** For example, the iodide $[Mo(CO)_5I][K[diglyme)_3]$ was first reported in 1959 (22, 23).

**[0047]** The introduction of the tetralkylammonium counter ions (Abel et. al., 1963) led to the stabilization of these complexes in the solid state allowing for the complete series of complexes $[Mo(CO)_5X][NR_4]$ to be prepared and characterized (X = Cl, Br, I). Cr and W cogeners of the fluoride analogue, $[Mo(CO)_5F]^-$ have been prepared by use of KF and crown-ethers (24, 25).

**[0048]** A slight modification of Abel's method, reported in 1985 (26), using more accessible solvents and lower temperatures, was found appropriate for the preparation of compounds of Formula I. This method consists of refluxing mixtures of $Mo(CO)_6$ and the appropriate tetraalkylammonium halide (X = Cl, Br, I) in THF and precipitation of the compounds by sequential cooling and addition of diethyl ether as depicted in equation (1).




(1)

**[0049]** This preparation resulted in high yields (approximately 90-95%).

**[0050]** Compounds of Formula I can also be prepared via halide replacement of photochemically generated $[Mo(CO)_5L]$ complexes with labile ligands (*e.g.*, L = $Me_3N$, NCMe, THF, $Et_2S$).

## Therapeutic Uses of the Compounds of Formula I

**[0051]** In one embodiment, a compound of Formula I exhibits a therapeutic effect in whole or in part due to the generation of free carbon monoxide. Carbon monoxide can be released from a compound of Formula I either by a spontaneous process or by a metabolic process, *i.e.*, with the involvement of one or more enzymes. The release of CO from the compound is in some embodiments assisted by donor molecules within an animal, such as water, proteins, or nucleotides.

[0052] In one embodiment, the compounds of Formula I release CO at specific sites in an animal, such as inflamed tissues or pre-atherosclerotic lesions of an artery. In another embodiment, the compounds of Formula I preferentially release CO in the presence of a reactive oxygen species that is generated at an inflammatory site or in an atherosclerotic lesion.

[0053] In one embodiment, compounds of Formula I are TNF inhibitors. In another embodiment, Compound I.1 is a TNF inhibitor. In one embodiment, compounds of Formula I are useful for the treatment of a disease known or suspected to be initiated or promoted by TNF, and are useful for the treatment of inflammatory diseases.

**Treatment or Prevention of Inflammatory Diseases**

[0054] The compounds of Formula I can be used to treat or prevent an inflammatory disease. Inflammatory diseases can arise where there is an inflammation of the body tissue. Examples of inflammatory diseases treatable or preventable using the compounds of Formula I, include, but are not limited to, transplant rejection; chronic inflammatory disorders of the joints, such as arthritis, rheumatoid arthritis, osteoarthritis and bone diseases associated with increased bone resorption; inflammatory bowel diseases such as ileitis, ulcerative colitis, Barrett's syndrome, and Crohn's disease; inflammatory lung disorders such as asthma, adult respiratory distress syndrome (ARDS), and chronic obstructive airway disease; inflammatory disorders of the eye such as corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic inflammatory disorders of the gum, such as gingivitis and periodontitis; tuberculosis; leprosy; inflammatory diseases of the kidney such as uremic complications, glomerulonephritis and nephrosis; inflammatory disorders of the skin such as sclerodermatitis, psoriasis and eczema; inflammatory diseases of the central nervous system, such as chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and viral or autoimmune encephalitis; autoimmune diseases such as diabetes mellitus, immune-complex vasculitis, systemic lupus erythematosus (SLE); inflammatory diseases of the heart such as cardiomyopathy, ischemic heart disease hypercholesterolemia, and atherosclerosis; as well as inflammation resulting from various diseases such as preeclampsia, chronic liver failure, brain and spinal cord trauma, and cancer. The compounds of Formula I can also be used to treat or prevent the progression of an

inflammatory disease and/or to reduce the symptoms of the inflammatory disease. In one embodiment, the compounds of Formula I are useful for treating or preventing pain associated with an inflammatory disease.

**[0055]** The inflammatory disease treatable or preventable by administration of an effective amount of a compound of Formula I can also be a systemic inflammation of the body. Examples of systemic inflammation include but are not limited to, gram-positive or gram-negative shock, sepsis, septic shock, hemorrhagic or anaphylactic shock, or SIRS.

**[0056]** In one embodiment, the inflammatory disease is circulatory shock, sepsis, systemic inflammatory response syndrome, hemorrhagic shock, cardiogenic shock, or systemic inflammation.

**[0057]** In one embodiment, a compound of Formula I can be used to treat or prevent an inflammatory skin disease. In one embodiment, the inflammatory skin disease is contact dermatitis, erythema, or psoriasis.

**[0058]** In one embodiment, the inflammatory disease is rheumatoid arthritis. In one embodiment, the inflammatory disease is juvenile idiopathic arthritis, psoriatric arthritis, or osteoarthritis. In another embodiment, the inflammatory disease is an inflammatory disease of the lung, including asthma and chronic obstructive pulmonary disease (COPD); an inflammatory disease of the skin, including psoriasis and contact dermatitis; an inflammatory disease of the intestinal tract, including inflammatory bowel disease, Crohn's disease, and ulcerative colitis; or an inflammatory disease of the liver, including viral hepatitis and autoimmune hepatitis. In one embodiment, the disease is a chronic inflammatory disease such as rheumatoid arthritis. In another embodiment, the inflammatory disease is a disease associated with a chronic inflammatory reaction, such as atherosclerosis or Alzheimer's disease; or with ischemia/reperfusion injury, such as myocardial infarction, stroke or organ transplantation. In one embodiment, the inflammatory disease is an infectious disease such as septic shock.

## Therapeutic Administration

**[0059]** In one embodiment, compounds described herein can be formulated into pharmaceutical compositions together with pharmaceutically acceptable carriers for oral administration in solid or liquid form, or for intravenous, intramuscular, subcutaneous, transdermal, or topical administration. In one embodiment, the compound is formulated with a pharmaceutically acceptable carrier for oral administration.

**[0060]** Pharmaceutically acceptable carriers for oral administration include capsules, tablets, pills, powders, troches, and granules. In the case of solid dosage forms, the carrier can comprise at least one inert diluent such as sucrose, lactose or starch. Such carriers can also comprise additional substances other than diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, troches and pills, the carrier can also comprise buffering agents. Carriers, such as tablets, pills and granules, can be prepared with enteric coatings on the surfaces of the tablets, pills or granules. Alternatively, the enteric coated compounds can be pressed into tablets, pills, or granules. Pharmaceutically acceptable carriers include liquid dosage forms for oral administration, *e.g.*, emulsions, solutions, suspensions, syrups and elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring agents.

**[0061]** Pharmaceutically acceptable carriers for topical administration include DMSO (dimethyl sulfoxide), alcohol or propylene glycol that can be employed with patches or other liquid retaining material to hold the medicament in place on the skin. Carriers based on nanoparticles and nanoencapsulates are also convenient for the protection of the active principle and its slow release in the organism or specific tissues.

**[0062]** Pharmaceutically acceptable carriers for intravenous administration include solutions containing pharmaceutically acceptable salts or sugars.

**[0063]** Pharmaceutically acceptable carriers for intramuscular or subcutaneous injection include salts, oils, or sugars.

**[0064]** Carriers such as solvents, water, buffers, alkanols, cyclodextrins and aralkanols can be used. Other auxiliary, non-toxic agents may be included, for example, polyethylene glycols or wetting agents.

**[0065]** Controlled delivery of drugs into the organism is important, especially for drugs that have undesired toxic effects if present systemically or at high local concentrations. CO release can be toxic at high concentrations. For certain applications, a slow release of CO in the blood or in specific target tissues is desirable. Encapsulation within host molecules that are non-toxic is one way to achieve a sustained release of active drugs in the organism. This strategy minimizes the undesired effects that may result from abrupt increases in the concentration and/or availability of a potentially toxic drug.

**[0066]** Cyclodextrins are well known hosts for many drugs and organic molecules and recently have been applied to host organometallic molecules and enhance their delivery through physiological barriers or membranes. In this respect, cyclodextrin has been found to

be beneficial for increasing delivery of lipophilic drugs at the skin barrier. (28) Cyclodextrin mediated supramolecular arrangements protect organometallic molecules for prolonged time periods and mask their reactivity, thereby increasing their selectivity towards specific reagents. The hydrophobic part of carbonyl complexes, as those exemplified under Formula I, fit inside β- or γ-cyclodextrin, or similar structures, with the CO groups facing the reaction medium and the organic ligands buried in the cavity. The resulting reduction in reactivity allows for the extension of the range of therapeutic CO-releasing complexes to cationic and anionic ones. Such charged complexes are more reactive and lose CO faster than the neutral ones when unprotected.

**[0067]** Liposomes and other polymeric nanoparticle aggregates are also useful carriers to target the delivery of CO-releasing organometallic complexes and the combined use of cyclodextrins with such aggregates has been considered as a very promising possibility for drug release. (29)

**[0068]** Mesoporous materials are chemically inert three dimensional molecules with infinite arrays of atoms creating channels and cavities of well defined pore size. These molecules are well suited to host organic and organometallic molecules in their pores. In the presence of biological fluids, smaller molecules undergoing acid-base and/or polar interactions with the inner walls of the pores slowly displace the included drugs, resulting in a controlled delivery of the active principle. Such aggregates have been prepared from M41S materials using organometallic molecules. Examples include MCM-41 (linear tubes) and MCM-48 (cavities and pores).

**[0069]** Hosting of compounds of Formula I by cyclodextrin, liposomes, other polymeric nanoparticles, or mesoporous materials can achieve sustained release of CO in vitro.

**[0070]** The pharmaceutically acceptable carriers and compounds described herein can be formulated into unit dosage forms for administration to an animal. The dosage levels of active ingredients (*i.e.*, compounds described herein) in the unit dosage can be varied so as to obtain an amount of active ingredient that is effective to achieve a therapeutic effect in accordance with the desired method of administration. The selected dosage level therefore mainly depends upon the nature of the active ingredient, the route of administration, and the desired duration of treatment. If desired, the unit dosage can be such that the daily requirement for an active compound is in one dose, or divided among multiple doses for administration, *e.g.*, two to four times per day.

**[0071]** In one embodiment, the compounds are administered orally once a day. The compounds described herein generate CO after administration to the body. Although CO is

generated preferentially at the sites of inflammation, some of the CO generated will bind to hemoglobin in red blood cells. Thus, dose-finding studies can be guided by measurement of carboxyhemoglobin (COHb) levels in the blood. Methods for the measurement of COHb levels in the blood are known in the art. In normal healthy humans, COHb levels are about 0.5% in healthy nonsmokers and up to 9% in smokers. In one embodiment, the dose level of the compounds described herein is such that no significant rise in COHb levels is observed. However, in some applications, a transient rise in COHb levels up to 10% may be tolerated. This level of COHb is not associated with any symptoms.

**[0072]** In one embodiment, a compound described herein can be administered in a dosage ranging between about 5 mmol/day and about 25 mmol/day, including about 6 mmol/day, about 7 mmol/day, about 8 mmol/day, about 9 mmol/day, about 10 mmol/day, about 11 mmol/day, about 12 mmol/day, about 13 mmol/day, about 14 mmol/day, about 15 mmol/day, about 16 mmol/day, about 17 mmol/day, about 18 mmol/day, about 19 mmol/day, about 20 mmol/day, about 21 mmol/day, about 22 mmol/day, about 23 mmol/day, or about 24 mmol/day, depending on the nature of the CO containing compound and its molar CO content.

**[0073]** In one embodiment, the invention provides the use of a compound of Formula I for the preparation of a medicament for inhibiting tumor necrosis factor production in an animal.

**[0074]** In one embodiment, the invention provides the use of a compound of Formula I for the preparation of a medicament for inhibiting TNF production in a cell.

**[0075]** In one embodiment, the invention provides the use of a compound of Formula I for the preparation of a medicament for treating or preventing an inflammatory disease in an animal.

**Examples**

**Example 1: Preparation of Compounds I.1 – I.2**

**[0076]** The general preparation and characterization of compounds of Formula **I** has been described by Wilkinson, *et al.* in the references. (28, 29)

**Compounds I.1, I.2 and I.6** are described and characterized in E. W. Abel, I. S. Butler and J. G. Reid, J. Chem. Soc., 2068 (1963). (27) We have, however, prepared them according to the modification introduced by Burgmayer and Templeton for the preparation of Compound I.3 (see Example 2). (26) The detailed preparation of Compound I.1 is given.

Preparation of Compound I.1:

A solution containing $Mo(CO)_6$ was prepared by dissolving 6.60 g (25.00 mmol) and 6.70g (31.9 mmol) of $Et_4NBr$ in 75 ml of THF. The mixture was refluxed for 2 hours, 30 minutes (Temp. = 85-90°C). Afterwards, the solution was immediately filtered (yellow solution) and half the solvent was evaporated under vacuum. A precipitate started to form and 60 ml of hexane were added to the solution to induce more precipitation. The schlenk tube was kept at -30°C for 1 hour. After that time, the solution was filtered and the yellow compound obtained was dried in vacuum. Yield: 89%. I.R.(KBr) (ν C≡O)($cm^{-1}$): 2069 (S), 1912 (S); 1871 (S); S=strong. Elemental Analysis $C_{13}H_{20}BrMoNO_5$:= 446.1496. % experimental (% calculated): C 34.88 (35.00); H 4.82 (4.52); N 3.06 (3.14)

Preparation of Compound I.2

Compound I.2 was prepared as described above in the preparation of Compound I.1. As will be recognized by those of skill in the art, other compounds described herein can be made similarly using the appropriate tetraalkylammonium halide.

Elemental (C, H, N) analysis confirmed the expected stoichiometry and spectroscopic data (IR, UV/vis, and NMR) were in agreement with those reported in (27) for Compound I.1.

**Example 2: Preparation of Compound I.3**

Compound I.3 was made as described in Burgmayer and Templeton. (26)

$Mo(CO)_6$ (1.50 g; 5.7 mmol) and $Et_4NI$ (1.52 g; 5.9 mmol) were put in a schlenk and 20 ml of THF were added. The suspension was refluxed for 130 minutes. The yellow solution was filtered hot to discard traces of white solid, and then concentrated to half its volume. Hexane was added, and the yellow solid, which precipitated immediately, was filtered and dried under vacuum to yield 2.70 g (96%) of pure compound.

IR (KBr pellet), $cm^{-1}$: 2072 (m), 1909 (s), 1872 (s).

Elemental Analysis Calculated for $C_{13}H_{20}NO_5IMo$: C, 31.66; H, 4.09; N, 2.84. Found: C, 32.07; H, 3.98; N, 2.85.

**Example 3: Spontaneous CO release.**

These studies were conducted in the apparatus shown in Figure 1. CO detection was carried out by Gas Chromatography using a thermal conductivity (TCD) detector for the quantification of CO and $CO_2$. The experiments were done under an initial atmosphere of reconstituted air, free of CO and $CO_2$. The medium used was RPMI with 10% Fetal Bovine

Serum. The suspension of Compound I.1 in RPMI/FBS or water was magnetically stirred and its temperature was kept at 37°C by using a thermostated circulating bath. Samples were withdrawn with gas-tight Hamilton syringes after homogenization of the head-space at given time intervals, preferably 2 hours, 4 hours and 6 hours. No attempts were made to quantify the CO gas remaining dissolved which, at this temperature, is very small due to the very low solubility of CO and the small total volume of solution used (3 mL). The volumes of CO released are usually in the range between 0.5 -3 mL. Due to the low solubility of Compound I.1 in water and RPMI, the CO release experiments were carried out on suspensions with the following amounts of Compound I.1: 2.4-3.5 mg Compound I.1/ml RPMI; 5.9 mg Compound I.1/ml $H_2O$ (pH = 2.13); 5.8 mg Compound I.1/ml $H_2O$ (pH = 8.3); 4.6 mg Compound I.1/ml olive oil. The amount of CO released (in equivalents of CO) is given in Table 1.

**Table 1:** Equivalents of CO released from suspensions of compounds of Formula I in different media, at 37°C in the dark. (numbers are averages)

| Compound | Time of reaction | RPMI | $H_2O$ pH = 2.13 | $H_2O$ pH = 8.3 | Olive oil |
|---|---|---|---|---|---|
| **Compound I.1** | 2 hours | 1.82 | 0.98 | 1.24 | 0.08 |
| | 4 hours | 2.16 | 1.00 | 1.03 | 0.25 |
| | 6 hours | 2.27 | 0.93 | 0.98 | 0.53 |
| **Compound I.6** | 2 hours | 0.42 | Not tested | Not tested | Not tested |
| | 4 hours | 1.00 | | | |
| | 6 hours | 1.25 | | | |

As a possible result of the use of suspensions, the number of CO equivalents released in RPMI varied slightly. As an example of the possible variations, the average of eight independent assays done with suspensions of Compound I.1 is given in Table 2.

**Table 2**: Equivalents of CO released by Compound I.1 in suspension in RPMI at 37°C in the dark. Average from eight independent assays.

| Time/hours | CO equivalents (average ± standard deviation) |
|---|---|
| 0.5 | 0.64±0.11 |
| 1 | 1.56±0.13 |
| 2 | 1.82±0.01 |
| 3 | 2.42±0.38 |
| 4 | 2.16±0.13 |
| 5 | 2.43±0.39 |
| 6 | 2.27±0.21 |
| 7 | 2.51±0.00 |
| 24 | 2.40±0.00 |

**Example 4: CO Release in the Presence of Reactive Oxygen Species ("ROS") (*e.g.*, Hydrogen Peroxide ($H_2O_2$), tert-Butyl Hydroperoxide (t-BuOOH; TBHP) and Potassium Superoxide ($KO_2$))**

The studies were done using the same method and apparatus described in Example 7 with the following modifications: RPMI/FBS was replaced by double distilled water in the

experiments with $H_2O_2$ and TBHP and by tetrahydrofuran (THF) for the experiments with $KO_2$; the temperature was kept at 25°C. The concentration of Compound I.1 was approximately 1mM and the ratio of concentrations of $H_2O_2$, TBHP and $KO_2$ relative to Compound I.1 was 100:1. The amount of $CO_2$ generated was also measured in the same experiment to ascertain the concurrent oxidation of coordinated CO. TBHP was added from a 70% aqueous solution and $H_2O_2$ from a 30% aqueous solution. The results are given in Table 3.

**Table 3:** Equivalents of CO and $CO_2$ released with different ROS at 25°C in the dark.

| Compound | Time of reaction | TBHP | | $H_2O_2$ | | $KO_2$ | |
|---|---|---|---|---|---|---|---|
| | | CO | $CO_2$ | CO | $CO_2$ | CO | $CO_2$ |
| **Compound I.1** | **1h** | 2.51 | 0.51 | 1.08 | 0.41 | 1.94 | 0.00 |
| | **3h** | 3.77 | 0.95 | 1.49 | 0.80 | 3.22 | 0.00 |
| | **5h** | 3.94 | 1.07 | 1.46 | 0.90 | 2.54 | 0.00 |
| | **24h** | 3.93 | 1.13 | 1.43 | 0.89 | 2.29 | 0.00 |
| **Compound I.6** | **1h** | 0.48 | 0.00 | 1.31 | 0.14 | Not Tested | Not Tested |
| | **3h** | 1.75 | 0.24 | 3.04 | 0.44 | | |
| | **5h** | 3.51 | 0.55 | 3.20 | 0.51 | | |
| | **24h** | 4.29 | 0.99 | 1.91 | 0.35 | | |

**Example 5: Toxicity *in vitro***

The cell toxicity of Compound I.1 was tested with RAW264.7 cells using the MTT assay to ascertain cell viability. Cells were seeded at $10^5$ per well with different concentrations of Compound I.1 and incubated for two to 24 hours; cell viability was then determined by the MTT assay; cells were incubated for 1 hour with 1 mg/ml MTT in DMEM, the supernatant was discarded and formazan crystals were dissolved in 150 ml DMSO. The results are given in Figure 2 for 2, 4 and 24 hours of incubation.

**Example 6: Toxicity *in vivo***

Compound I.1 was dissolved in olive oil and administered to Sprague Dawley rats at a daily dose of 80 mg/kg for 20 days. At the end of the treatment the rats were anesthetized, blood was collected and organ samples were fixed in formalin for histological analysis. No signs of liver or kidney toxicity were observed. The serum values for glutamic oxalacetic transaminase (sGOT), glutamic pyruvic transaminase (sGPT), creatinine and urea were in the

normal range. Histologic analysis did not reveal any gross alterations in the liver, kidney, heart, and spleen.

**Example 7: CO release *in vivo***

Nine week old Balb/c mice with a body weight of about 20 g were injected by the intraperitoneal route with Compound I.1 dissolved in a propylene glycol-water mixture. Three doses (100, 25 and 6.25 mg/kg) were used. At various times after the administration of the Compound I.1 blood was collected and CO-hemoglobin levels were determined using an oximeter. The results were obtained after 0, 30, 120 and, in one case, 330 minutes are given in Figure 3. The results show an increase in CO levels during the first time interval, followed by a slow decline from peak CO-levels over the next few time intervals.

**Example 8: Inhibition of LPS-induced TNF Production in Mice**

**[0077]** The ability of Compound I.1 to inhibit TNF production was tested in mice according to the procedure of WO 98/38179. Eight week old, female Balb/c mice received intraperitoneal injections of Compound I.1 at different doses (3, 10 and 30 mg/kg) or vehicle (carboxymethylcellusose 0.5%, Tween80 0.5%) only. Thirty minutes later all mice received intraperitoneal injections of LPS 0111:B4 Sigma at a dose of 0.3 mg/kg. Ninety minutes after the injection of LPS, serum samples were collected and analyzed for TNF content by ELISA. The data are shown in Figure 4. These data show that Compound I.1 inhibited TNF production with an $ED_{50}$ of about 22 mg/kg.

**Example 9: Impact on Mortality in Mice after Injection of a Lethal Dose of LPS**

**[0078]** Seventeen eight week old Balb/c mice received one intraperitoneal injection of LPS at a dose of 10 mg/kg at time zero. One group of eight mice received four intraperitoneal injections of Compound I.1, each at a dose of 20 mg/kg, at 60 and 30 minutes before LPS and at 4 hours and 9 hours after LPS. A second group of 9 mice received four intraperitoneal injections of vehicle (carboxymethylcellulose 0.5%, Tween80 0.5%) at 60 and 30 minutes before LPS and at 4 hrs and 9 hrs after LPS. Survival of the mice was monitored for 168 hours. As shown in Figure 5, all nine vehicle treated mice were dead at 47 hours following LPS injection while three of the eight mice treated with Compound I.1 remained alive at 168 hours following LPS injection, at which time they were sacrificed. These data

demonstrate a significant inhibition of LPS-induced lethal effects of lipopolysaccharide by Compound I.1.

**Example 10: Treatment of Adjuvant Arthritis in Rats with Compound I.1**

**[0079]** Adjuvant arthritis was induced in 11 week old, outbred Wistar rats (376 – 400g) by a single intradermal injection into the subplanatar area of the right hind paw of 100 microliter of a 10 mg/ml suspension of mycobacterium butyricum in incomplete Freund's Adjuvant. The disease was induced in 3 groups of rats each consisting of 7 animals. Group 1 (control) did not receive any treatment. Groups 2 and 3 received daily applications of methylene chloride (positive control) (500 mg/kg), or Compound I.1 (80 mg/kg), respectively. Both compounds were administered in olive oil by oral gavage. Treatment was initiated at day 10 after disease induction when signs of arthritis began to appear in the injected footpad as well as in the contralateral footpad. The treatment lasted for 20 days until day 29 after disease induction. At day 20 of treatment, the control group was reduced by three rats with severe arthritis. These three rats were then treated with Compound I.1 for 10 days. All animals were evaluated daily by determination of body weight, foot pad volume (performed by a water displacement method using a plethysmometer, Ugo Basile, Comerio, Italy), ankle circumference (using a flexible measuring tape) and arthritic index that is based on levels of erythema and oedema of the entire paws and digits, number of joints involved, spondilosis, lesions on tail, movment capacity and infections (0 = normal, 1 = swelling and /or redness of injected paw; 4 = severe arthritis of the entire injected paw and digits ; +2 = 2 joints are involved; +3 = >2 joints are involved; +1 = infection of paws; +1 = tail lesions; +1 = movment incapacity; +1 = spondilosis). The sum of the parameters is calculated as an arthritis index with a maximum possible score of 11.

**[0080]** The results are shown in Figures 6, 7 and 8. Figures 6A-6B show the average left (Figure 6A) or right (Figure 6B) paw volume in rats of the control, positive control-treated and Compound I.1-treated groups. Figures 7A-7B show the average left (Figure 7A) or right (Figure 7B) paw circumference in rats of the control, positive control-treated and Compound I.1-treated groups. Figure 8 demonstrates the arthritis index in rats of the control, positive control-treated and Compound I.1-treated groups. Methylene chloride was used as a positive control in each instance. Methylene chloride generates CO when it is metabolized in the liver and has previously been shown to have beneficial effects in a rat arthritis model (US 2003/0068387). Compound I.1 at 80 mg/kg was superior to methylene chloride at 500 mg/kg

in all measured parameters. The three rats of the control group that were treated with Compound I.1 from day 20 on showed also signs of improvements after 10 days.

**Example 11**

Compound I.1 was administered intraperitonally to mice at a concentration of 100 mg/kg using propylene glycol/water ca.~ 2:1 as vehicle. The amount of COHb (carboxyhemoglobin) was monitored with an oximeter in blood samples withdrawn at 0, 30, 120 and 330 minutes after administration. The results are shown in Figure 9 and show a peaked level of CO after 30 minutes followed by a slow decline.

**Example 12**

Compound I.1 was encapsulated in methylated ß-cyclodextrin, 2,3,6-tri-O-methyl-ß-cyclodextrin, known in the art as TRIMEB, by a standard technique. The encapsulated Compound I.1@TRIMEB was administered intraperitonally to mice at a concentration of 30 mg/kg using phosphate buffered saline (PBS) as vehicle. The amount of COHb (carboxyhemoglobin) was monitored with an oximeter in blood samples withdrawn after 30, 60, 90 and 120 minutes after administration. The results are shown in Figure 10 and demonstrate a less intensive and slower release of CO in the encapsulated complexes with a more sustained profile.

**[0081]** While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

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# Claims

1. A method for inhibiting tumor necrosis factor (TNF) production in an animal in need thereof, comprising administering to the animal an effective amount of a compound of the Formula I:

$$[Mo(CO)_5Y]Q$$

**I**

wherein

Y is bromide, chloride or iodide; and

Q is $[NR^{1-4}]^+$; and

$R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

2. A method for inhibiting TNF production in a cell, comprising contacting the cell with a compound of the Formula I:

$$[Mo(CO)_5Y]Q$$

**I**

wherein

Y is bromide, chloride or iodide; and

Q is $[NR^{1-4}]^+$; and

$R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

3. A method for treating or preventing an inflammatory disease in an animal in need thereof, comprising administering to the animal an effective amount of a compound of the Formula I:

$$[Mo(CO)_5Y]Q$$

**I**

wherein

Y is bromide, chloride or iodide; and

Q is $[NR^{1-4}]^+$; and

$R^1$, $R^2$, $R^3$, and $R^4$ are each independently alkyl.

4. The method of claim 3, wherein Q is a tetraethylammonium cation, a tetra(n-butyl)ammonium cation, a tetra(n-propyl)ammonium cation, a tetra(i-propyl)ammonium cation or a tetramethylammonium cation.

5. The method of claim 3, wherein Q is a tetraethylammonium cation.

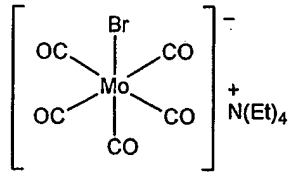
6. The method of claim 3, wherein $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_{12})$-alkyl.

7. The method of claim 3, wherein $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_8)$-alkyl.

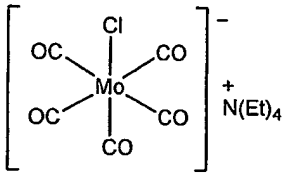
8. The method of claim 3, wherein $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_6)$-alkyl.

9. The method of claim 3, wherein $R^1$, $R^2$, $R^3$, and $R^4$ are $(C_1\text{-}C_4)$-alkyl.

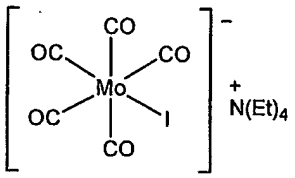
10. The method of claim 3, wherein the compound is one of the following compounds:



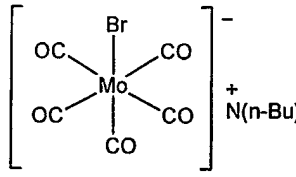

I.1



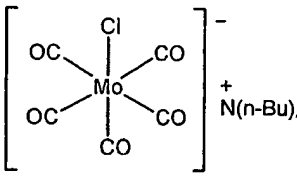

I.2



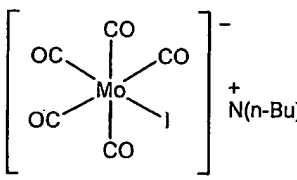

I.3



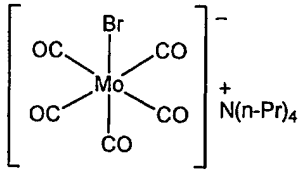

I.4



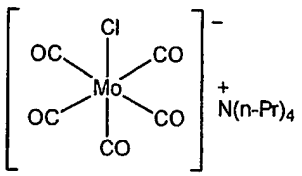

I.5



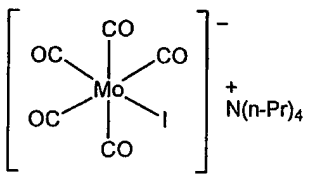

I.6




I.7




I.8

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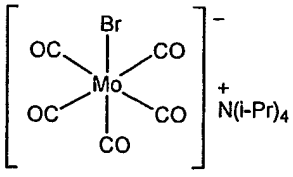


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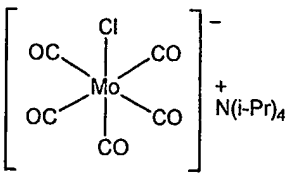
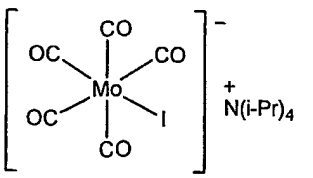
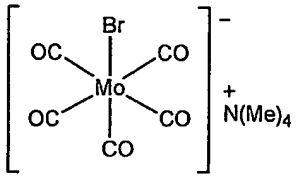


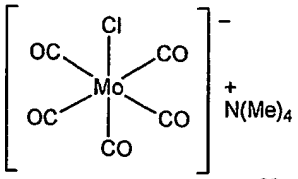

I.11




I.12

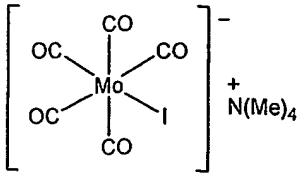



I.13



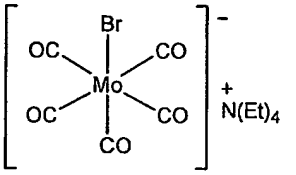

I.14

or

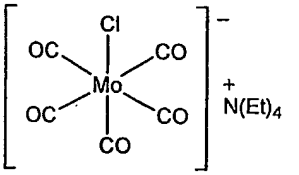



I.15

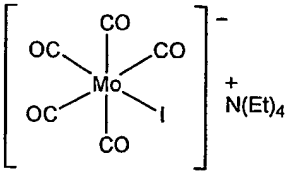
The method of claim 3, wherein the compound is one of the following compounds:




I.1

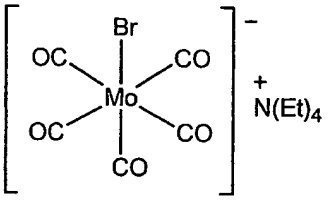



I.2




I.3

11.




12. The method of claim 3, wherein the compound is I.1

13. The method of claim 3, wherein the inflammatory disease is arthritis.

14. The method of claim 3, wherein the inflammatory disease is rheumatoid arthritis.

15. The method of claim 3, wherein the inflammatory disease is juvenile idiopathic arthritis, psoriatric arthritis or osteoarthritis.

16. The method of claim 3, wherein the inflammatory disease is asthma, chronic obstructive pulmonary disease, or an inflammatory lung disease.

17. The method of claim 3, wherein the inflammatory disease is ulcerative colitis, Crohn's disease, or an inflammatory bowl disease.

18. The method of claim 3, wherein the inflammatory disease is a disease associated with a chronic inflammatory reaction.

19. The method of claim 3, wherein the inflammatory disease is atherosclerosis or Alzheimer's disease.

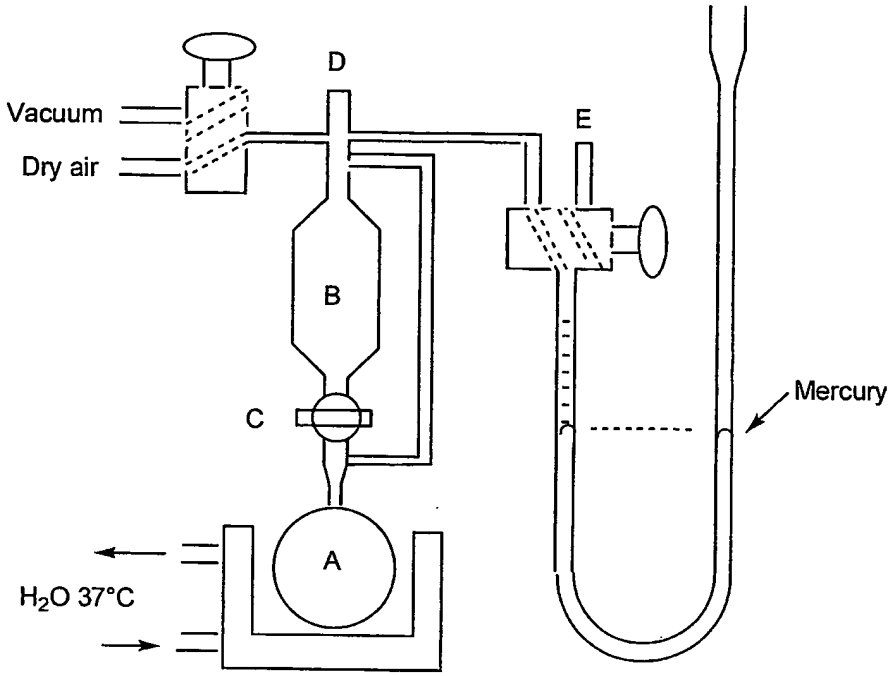
20. The method of claim 3, wherein the inflammatory disease is psoriasis, contact dermatitis or an inflammatory skin disease.

21. The method of claim 3, wherein the inflammatory disease is a disease associated with ischemia/reperfusion injury.

22. The method of claim 3, wherein the inflammatory disease is myocardial infarction, stroke or organ transplantation.

23. The method of claim 3, wherein the inflammatory disease is viral hepatitis, autoimmune hepatitis or an inflammatory disease of the liver.

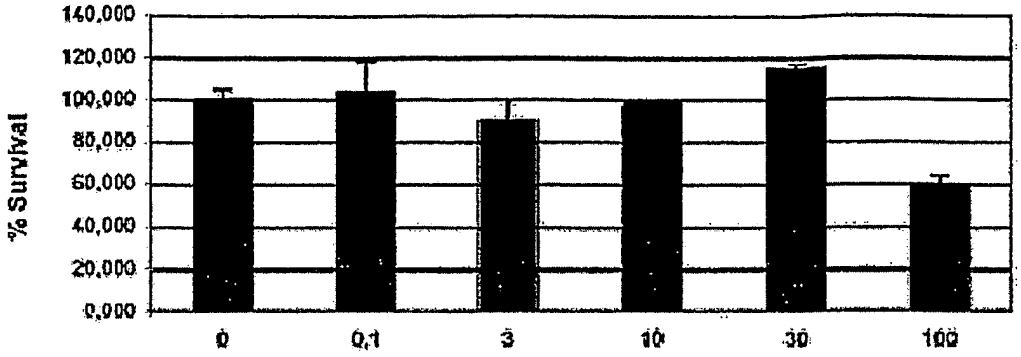
24. The method of claim 3, wherein the inflammatory disease is septic shock or an infectious disease.




**Operation**: The compound to be tested is charged in **A**; the system is evacuated and refilled with $CO_2$ free air; The medium is added via syringe, to **B** and at time 0 to **A**, after opening **C**. Volume of gas released is measured in the burette **E** which also serves to force gas homogenization within the system (up-down movement of the mercury); gas samples are withdrawn through septum **D**, after homogenizing and collecting all gas into chambers **A** plus **B** (constant volume and temperature.)

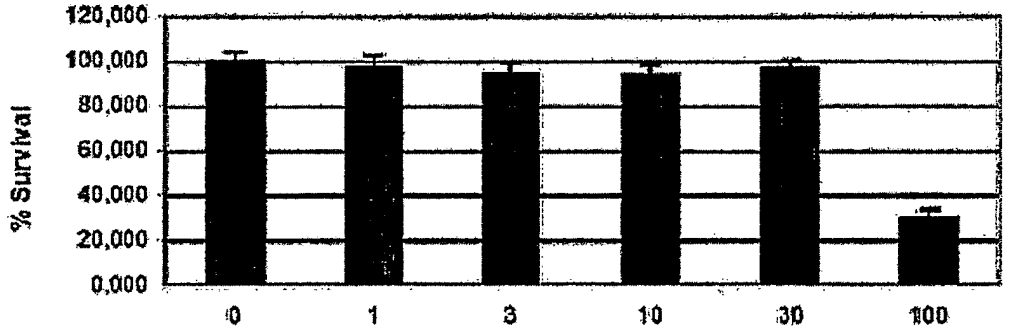
**Figure 1**

Toxicity of Compound I.1 in RAW264.7 cells (2 hours)



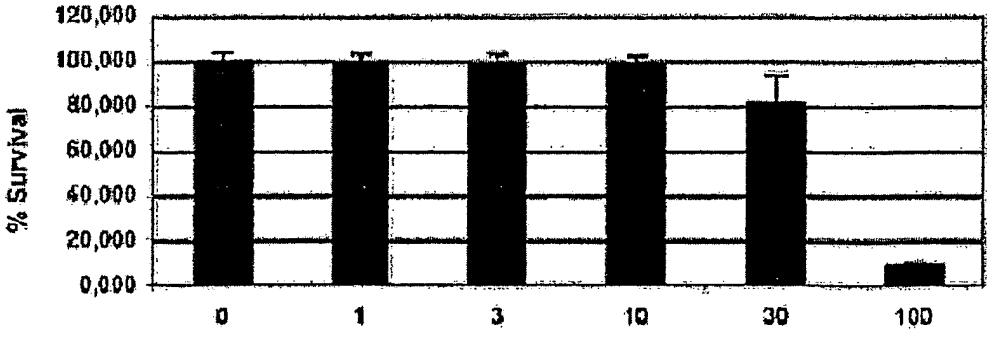

[Compound I.1] (μM ± standard deviation)

Toxicity of Compound I.1 in RAW264.7 cells (4 hours)




[Compound I.1] (μM ± standard deviation)

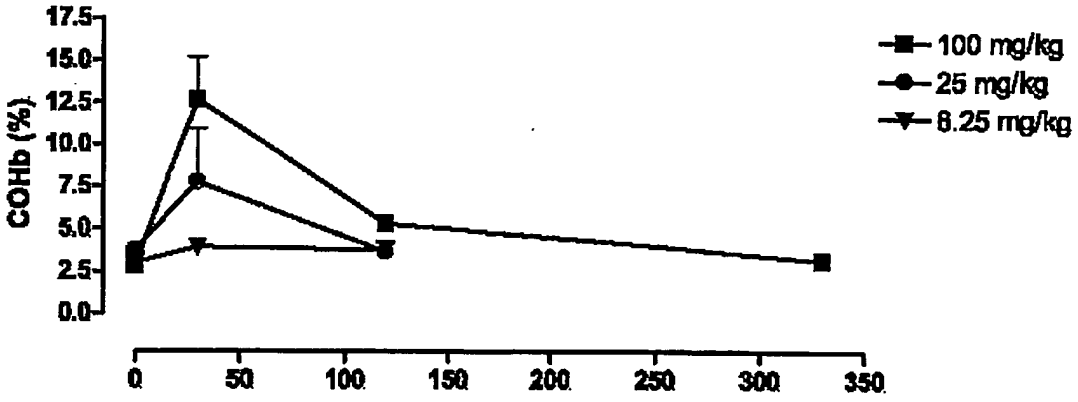
Toxicity of Compound I.1 in RAW264.7 cells (24 hours)




[Compound I.1] (μM ± standard deviation)

Figure 2

COHb induced by Compound I.1 administered i.p.




Time after administration of Compound I.1 (min)

Figure 3

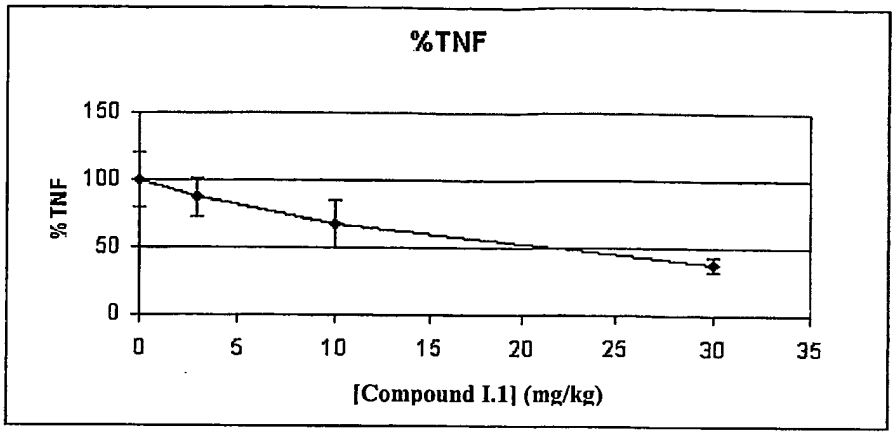



Figure 4

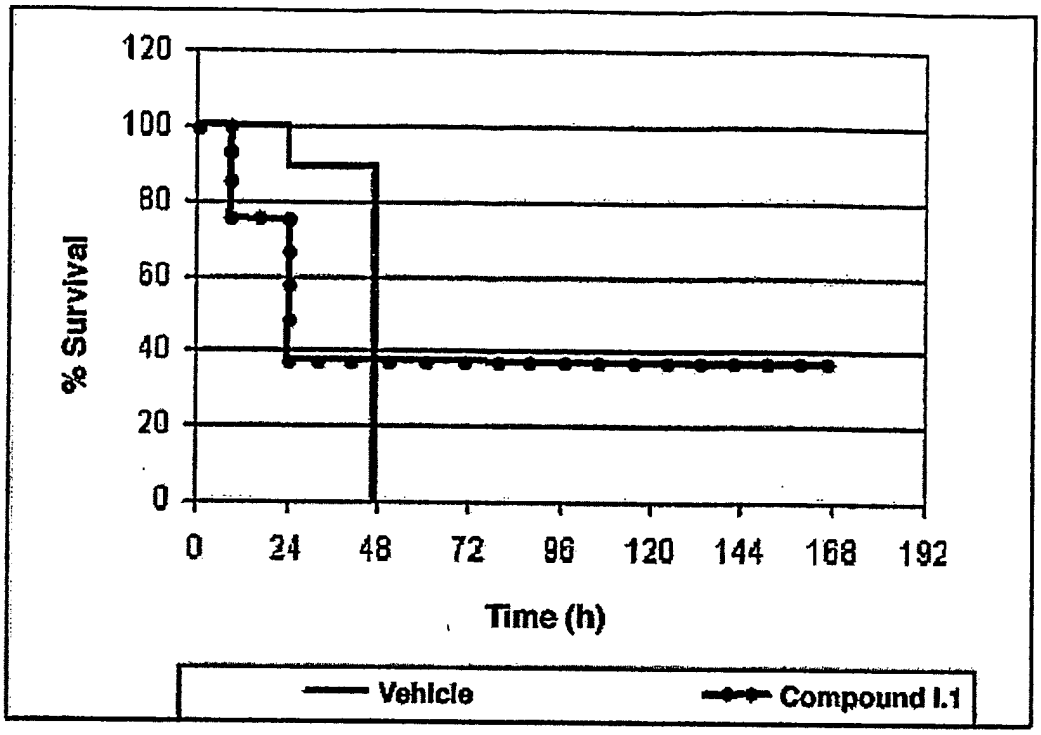

120
100
80
60
40
20
0
% Survival
0
24
48
72
96
120
144
168
192
Time (h)
Vehicle
Compound I.1

Figure 5

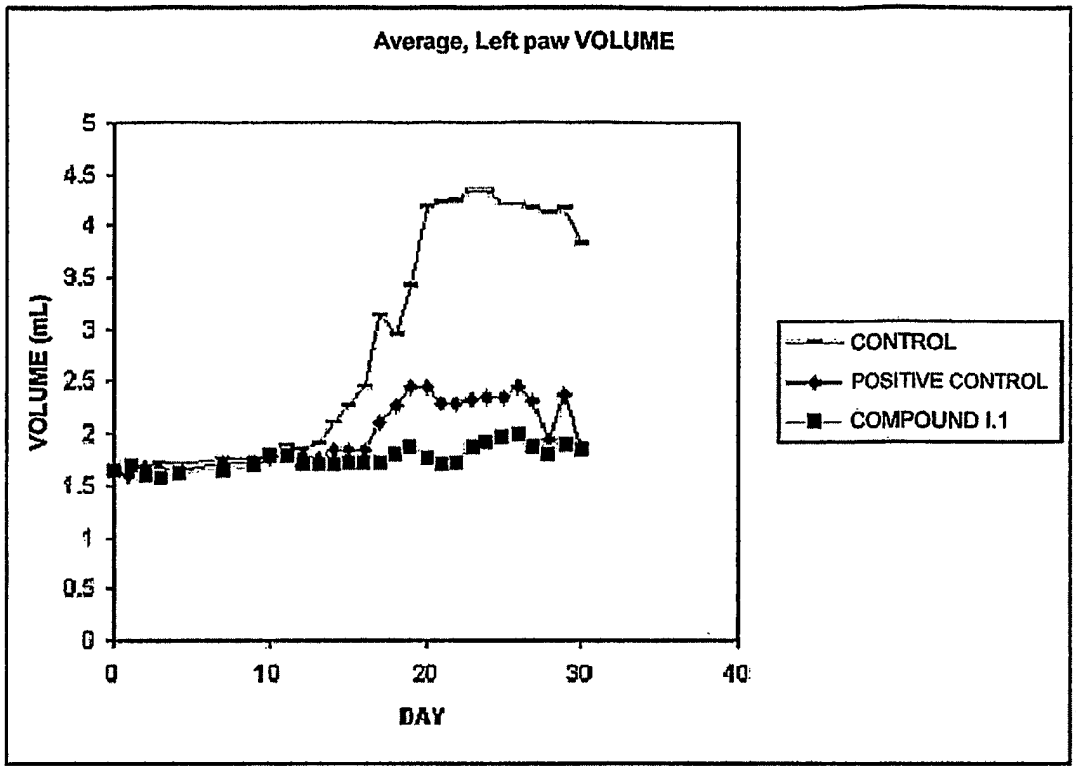

Average, Left paw VOLUME
VOLUME (mL)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
DAY
CONTROL
POSITIVE CONTROL
COMPOUND I.1

Figure 6A

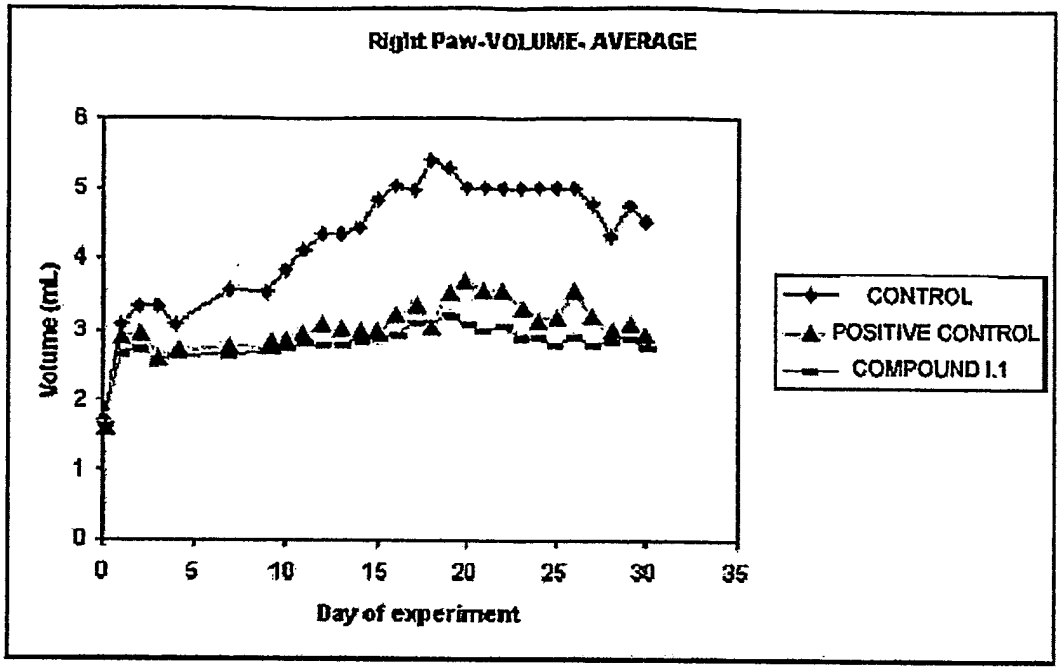

Right Paw-VOLUME- AVERAGE
Volume (mL)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
Day of experiment
CONTROL
POSITIVE CONTROL
COMPOUND I.1

Figure 6B

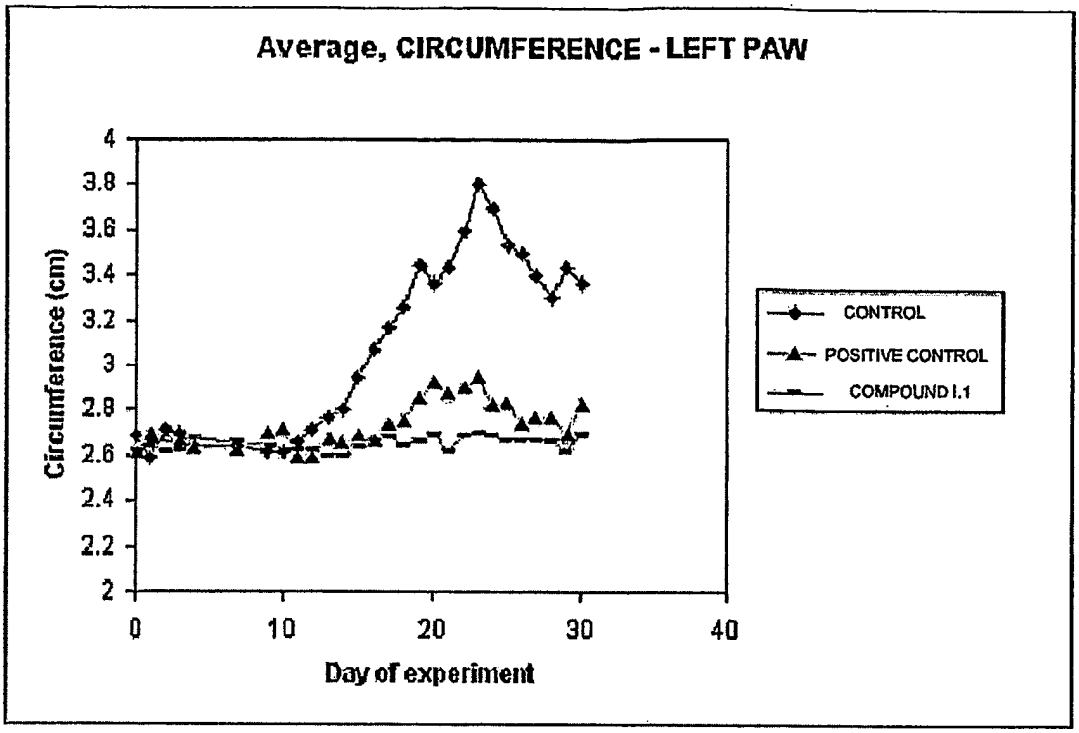

Average, CIRCUMFERENCE - LEFT PAW
Circumference (cm)
4
3.8
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
0
10
20
30
40
Day of experiment
CONTROL
POSITIVE CONTROL
COMPOUND I.1

Figure 7A

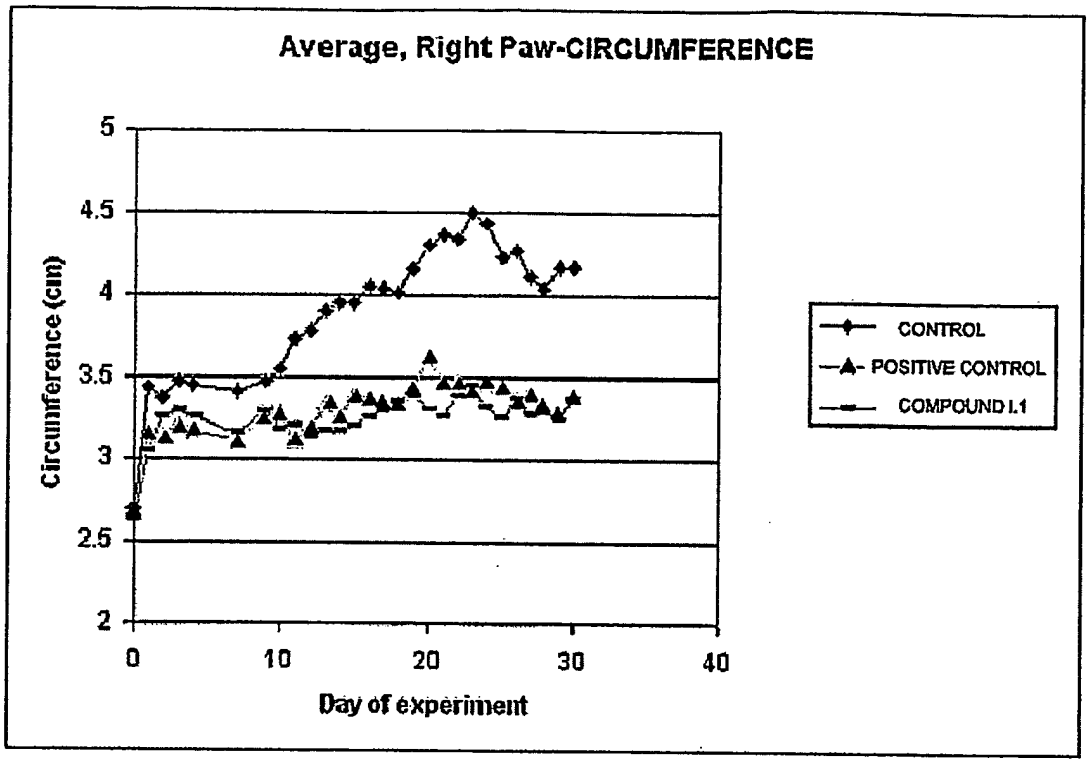

Average, Right Paw-CIRCUMFERENCE
Circumference (cm)
5
4.5
4
3.5
3
2.5
2
0
10
20
30
40
Day of experiment
CONTROL
POSITIVE CONTROL
COMPOUND I.1

Figure 7B

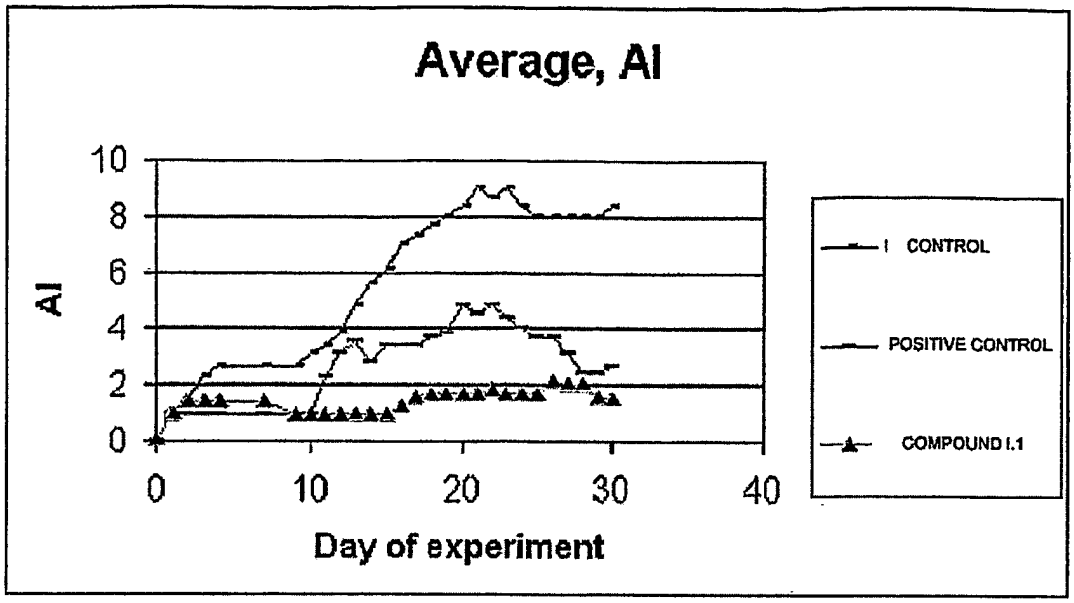

Average, AI
AI
10
8
6
4
2
0
0
10
20
30
40
Day of experiment
CONTROL
POSITIVE CONTROL
COMPOUND I.1

Figure 8

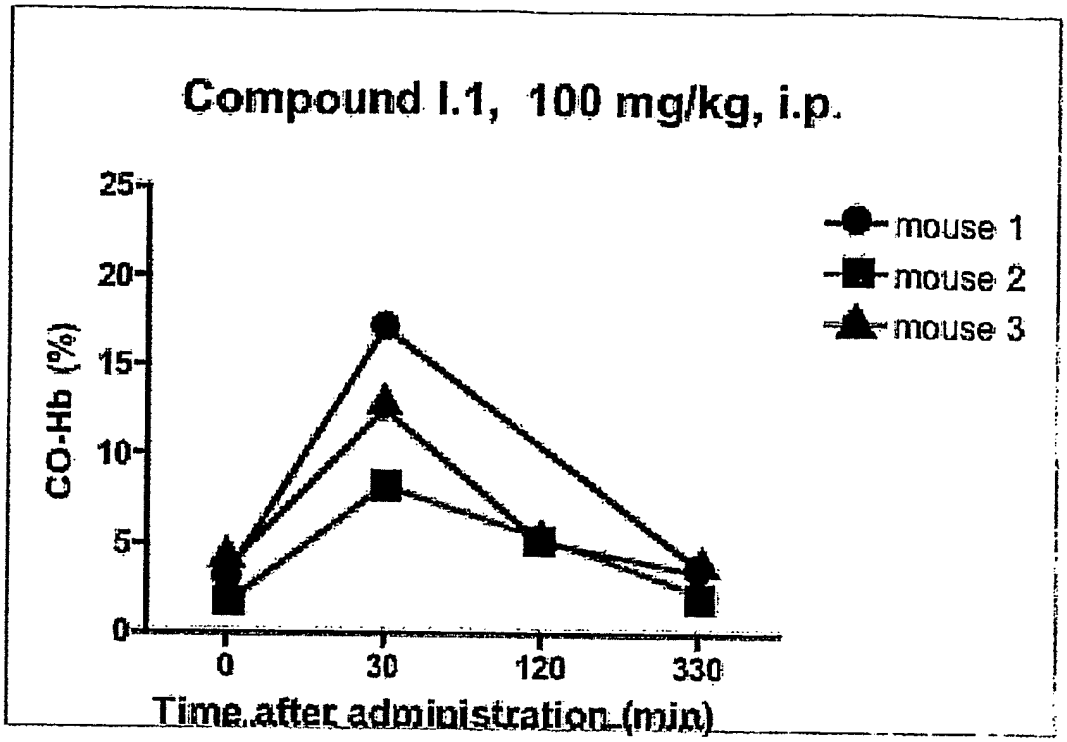

Compound I.1, 100 mg/kg, i.p.
CO-Hb (%)
25
20
15
10
5
0
0
30
120
330
Time after administration (min)
mouse 1
mouse 2
mouse 3

Figure 9

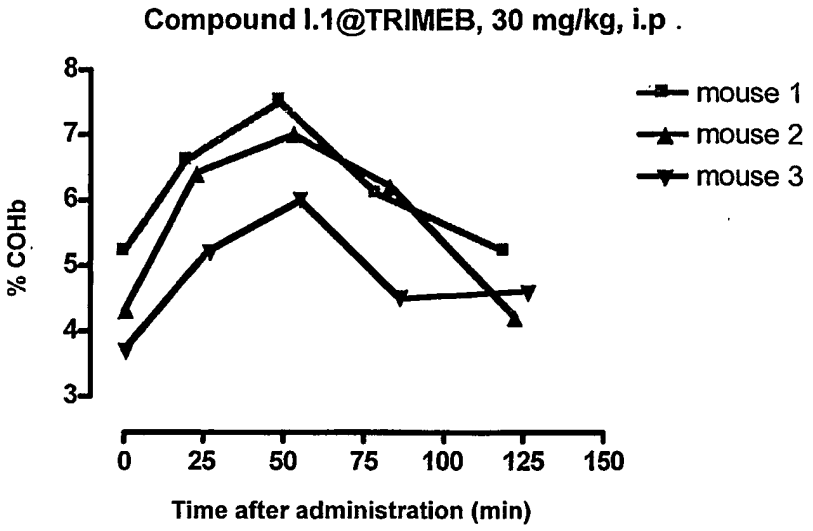

Compound I.1@TRIMEB, 30 mg/kg, i.p .
% COHb
8
7
6
5
4
3
0
25
50
75
100
125
150
Time after administration (min)
mouse 1
mouse 2
mouse 3

Figure 10

# INTERNATIONAL SEARCH REPORT

International application No
PCT/PT2006/000030

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K33/24 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,Y | WO 03/066067 A (HAAS WERNER [PT]; ROMAO CARLOS [PT]; ROYO BEATRIZ [PT]; FERNANDES ANA) 14 August 2003 (2003-08-14)<br>page 19<br>page 36<br>claims | 1-24 |
| X,Y | WO 02/092075 A (NORTHWICK PARK INST FOR MEDICA [GB]; UNIV SHEFFIELD [GB])<br>21 November 2002 (2002-11-21)<br>cited in the application<br>pages 9,13,17 | 1-24 |
| Y | WO 02/078684 A (SANGSTAT MEDICAL CORP [US]) 10 October 2002 (2002-10-10)<br>cited in the application<br>page 9 | 1-24 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 May 2007 | 18/05/2007 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Steendijk, Martin |

Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/PT2006/000030

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | OTTERBEIN L E ET AL: "Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway" NATURE MEDICINE, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 6, no. 4, April 2000 (2000-04), pages 422-428, XP002249546 ISSN: 1078-8956 abstract | 1-24 |
| A | US 3 278 570 A (GEOFFREY WILKINSON ET AL) 11 October 1966 (1966-10-11) cited in the application columns 1-2 | 1-24 |
| A | ABEL E W ET AL: "THE ANIONIC HALOPENTACARBONYLS OF CHROMIUM, MOLYBDENUM, AND TUNGSTEN" JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL SOCIETY. LETCHWORTH, GB, 1963, pages 2068-70, XP009083368 ISSN: 0368-1769 cited in the application abstract | 1-24 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/PT2006/000030

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 03066067 A | 14-08-2003 | AU 2003208525 A1 | 02-09-2003 |
| | | BR 0307420 A | 21-12-2004 |
| | | CA 2475209 A1 | 14-08-2003 |
| | | CN 1646140 A | 27-07-2005 |
| | | EP 1476168 A2 | 17-11-2004 |
| | | JP 2005519928 T | 07-07-2005 |
| WO 02092075 A | 21-11-2002 | CA 2447275 A1 | 21-11-2002 |
| | | CN 1561207 A | 05-01-2005 |
| | | EP 1399147 A2 | 24-03-2004 |
| | | JP 2004532244 T | 21-10-2004 |
| | | US 2003064114 A1 | 03-04-2003 |
| | | US 2006115542 A1 | 01-06-2006 |
| WO 02078684 A | 10-10-2002 | CA 2442457 A1 | 10-10-2002 |
| | | CN 1507348 A | 23-06-2004 |
| | | EP 1381354 A2 | 21-01-2004 |
| | | JP 2004526739 T | 02-09-2004 |
| | | MX PA03008820 A | 30-07-2004 |
| US 3278570 A | 11-10-1966 | NONE | |